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THE UNIVERSITY OF ALBERTA

EFFECT OF TANNINS IN RAPESEED MEAL ON ITS NUTRITIONAL VALUE FOR CHICKS

by

Ⓒ ZEKI YAPAR

A THESIS
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Effect of Tannins in Rapeseed Meal on its Nutritional Value for Chicks" submitted by Zeki Yapar, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

An investigation was carried out to study the effects of tannins in rapeseed meal on its nutritive value for chicks. Two experiments were conducted to study the effects of the tannins in rapeseed meal on its metabolizable energy and on the absorbability of its nitrogen.

The results obtained showed that extraction of tannins from rapeseed meal significantly increased its metabolizable energy content for chicks from 1,171 to 1,844 kcal per kg and from 1,253 to 1,766 kcal per kg at 2 and 6 weeks of age, respectively. Addition of tannins extracted from rapeseed meal to a ration in which soybean meal served as the sole source of protein significantly reduced its metabolizable energy value.

The absorption of nitrogen from rapeseed meal was not affected by removal of tannins from the meal. Nitrogen absorption from rapeseed meal and tannin-free rapeseed meal were 62.6 and 64.2% respectively when the meals were fed in combination with soybean meal and 79.7 and 80.4% respectively when fed as the sole source of nitrogen in the ration.

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INTRODUCTION

Rapeseed is a crop, well adapted to Canadian conditions. It is a good source of vegetable oil and, as a byproduct of oil extraction, provides a high-protein meal for livestock and poultry feeding. Analyses of the proximate principles of the meals indicates that, in general, rapeseed meal is comparable to other plant protein meals.

In modern methods of oil extraction, meals are subjected to less heat during processing and the amount of oil left in the meal is greatly reduced as compared to expeller meals. As a result of this procedure, the meals are of much better quality than those produced a few years ago. Examination of essential amino acid composition of modern rapeseed meals indicates that they should be generally equivalent to other plant protein supplements.

Use of rapeseed meal for feeding poultry has been limited because it contains factors that may affect thyroid size, growth rate, reproduction and livability of animals. One of the factors that may be involved is the presence of tannins in the meal since it has been reported that the addition of tannins to poultry rations may reduce rate of growth and may affect utilization of energy and protein.

The present study was designed to determine the effect of tannins in rapeseed on the metabolizable energy content of the meals and to assess the influence of the tannins on nitrogen absorbability.

REVIEW OF LITERATURE

Chemistry of tannins

Tannins are polyphenolic compounds with molecular weights ranging from 500 to 3000 g that are found widely distributed in plants (White, 1956). According to Haslam (1966) plant material also contains simpler polyphenols (monomeric flavonoids and derivatives of hydroxycinnamic acid) which because of their low molecular weight are not considered to be tannins.

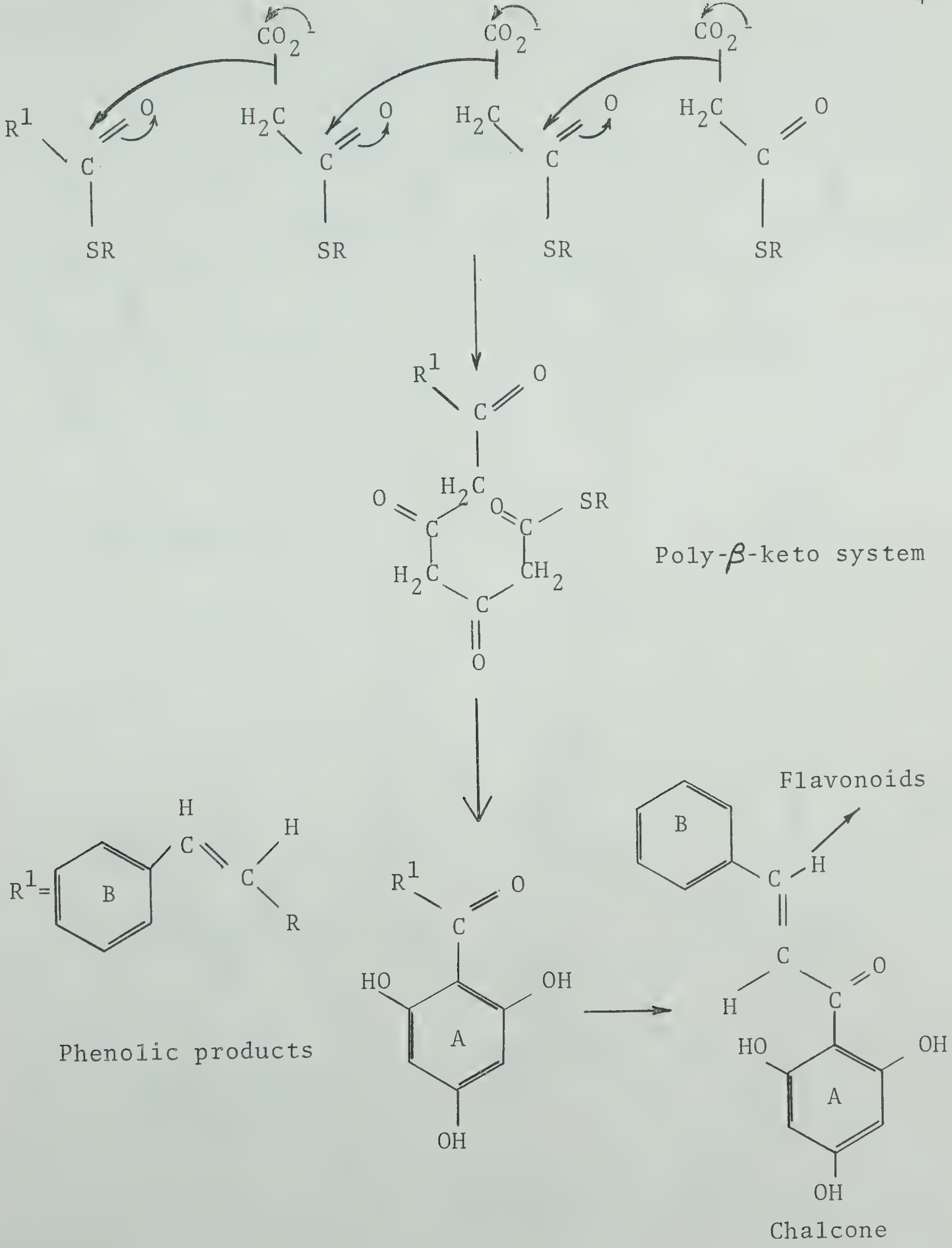
Freudenberg (1920) classified tannins into the hydrolysable and condensed tannins basing the distinction on their reaction to acids. The hydrolysable tannins, which have a polyester structure, are readily hydrolysed by acids or enzymes to yield sugars or related polyhydric alcohols and phenol carboxylic acids. Depending on the nature of the latter, a subdivision into gallotannins and ellagitannins is also usually made. Thus on hydrolysis, the gallotannins yield gallic acid while the ellagitannins yield hexahydroxydiphenic acids in addition to the sugar or alcohol moiety. The alcoholic portion of the hydrolysable tannins has most often been found to be D-glucose, although in some cases other sugars have been isolated from the tannin core. The condensed tannins, in contrast, do not readily break down with acids; instead they undergo progressive polymerization to amorphous phlobaphens or tannin reds.

The metabolism of phenyl propanoid compounds in vascular

plants is connected not only with lignin biosynthesis but also with the biosynthesis of other substances (Haslam, 1966). Birch (1962) suggested that condensation of one molecule of cinnamic acid or related $C_6.C_3$ component with three malonate molecules would yield a chalcone as the starting compound of the flavonoid biosynthesis. This concept has received support by Birch and Donovan (1953) in studies on the biosynthesis of different flavonoid compounds. These workers found that isotopically labelled acetate precursors, were incorporated into the A-ring while phenyl propanoid precursors were incorporated into the B-ring of the flavonoids. They were presumed to arise via the shikimic-prephenic acid pathway shown in Equation 1.

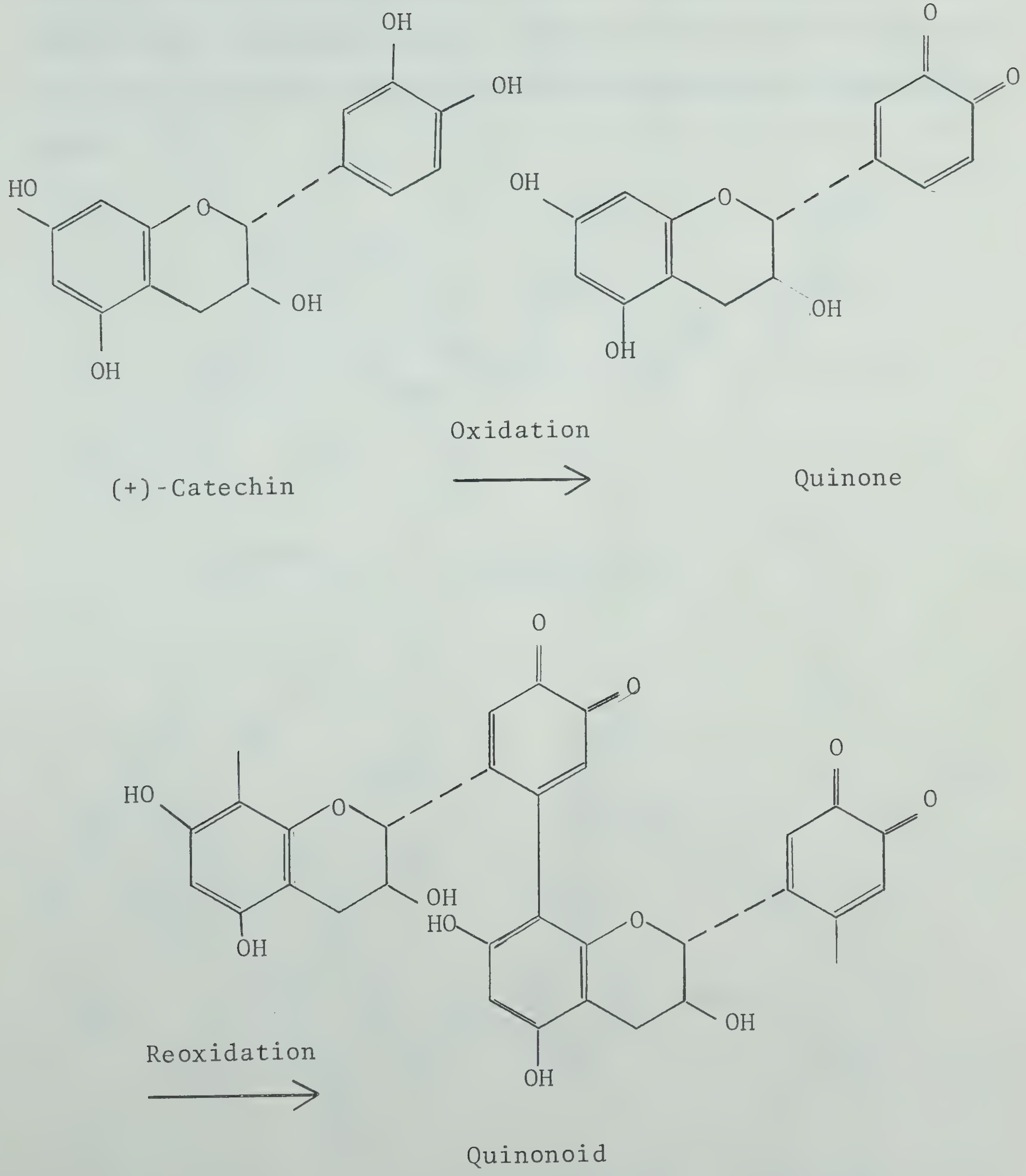
The biosynthesis of condensed tannins is attributed to a process of condensation or polymerization of monomeric flavan -3, 4-diol or flavan -3-ol precursors (Roux and Paulus, 1961). While the weight of existing evidence, according to Haslam (1966) favours theories of condensed tannin formation by enzymatic condensation of flavan -3, 4-diols; Hathway and Seakins (1955, 1957) proposed enzymatic oxidation of catechins, (Equation 2) as an alternative mechanism. Considering the increasing significance of phenol oxidation mechanisms in the biogenesis of a wide spectrum of natural products the above mechanism for catechin-tannin formation may hold significance in relation to the composition of condensed tannins (Roux, 1968).

A scheme for the biosynthesis of gallic acid (Roux,



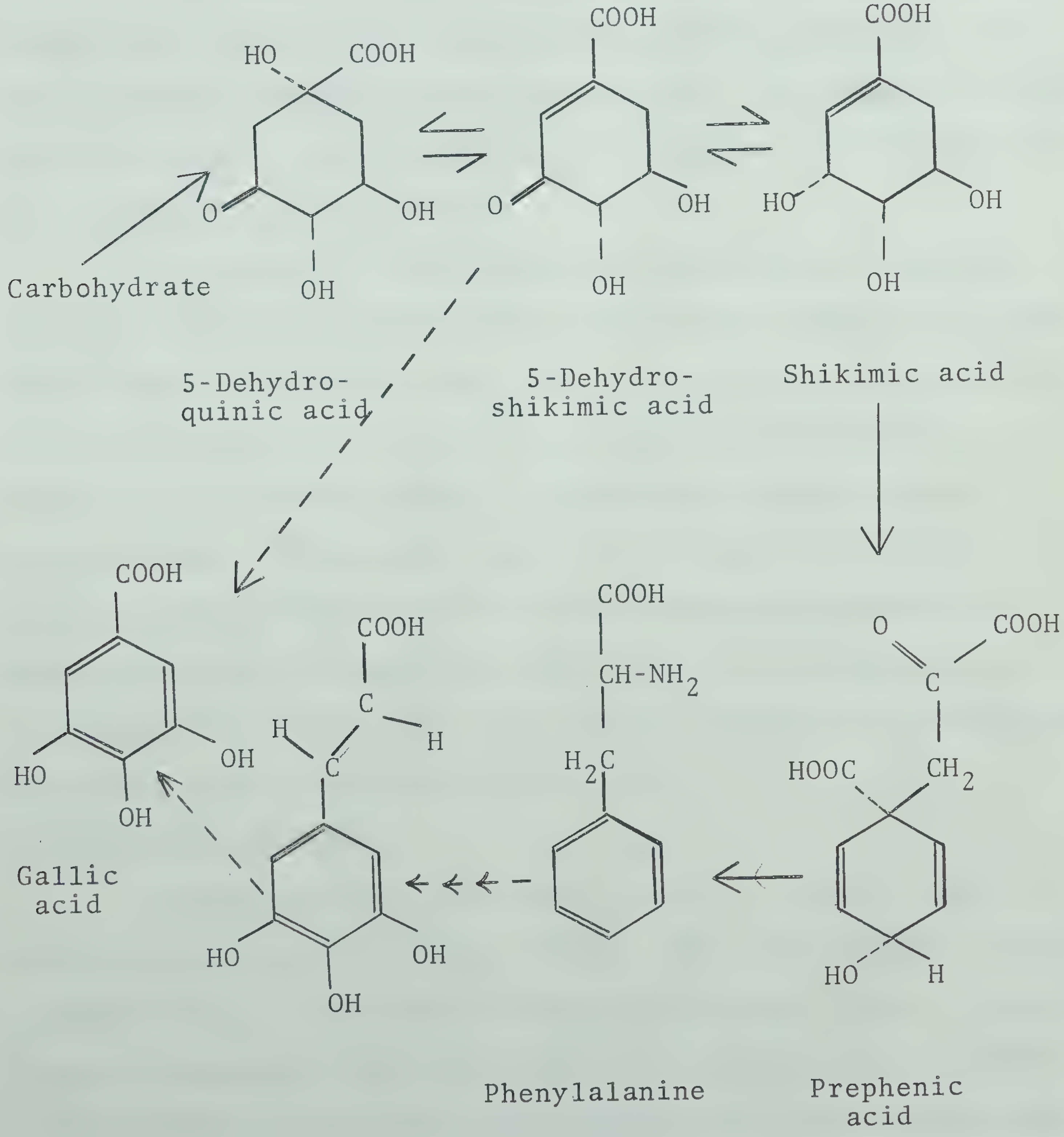
Equation 1. Biosynthesis of flavonoids

R^1 = acetate, SR = coenzyme A.



Equation 2. Biosynthesis of condensed tannins

1968) has been proposed (Equation 3). Biosynthesis of free gallic acid from glucose as a carbon source by lower organisms has been demonstrated for a strain of Aspergillus (Sasaki and Takao, 1953).



Equation 3. Biosynthesis of gallic acid (Roux, 1968)

Zenk (1964) concluded that gallic acid is derived from phenylalanine through oxidative degradation. Evidence at variance with the above conclusion has been obtained by Conn and Swain (1961) from studies in which acetate, glucose and phenylalanine served as precursors of gallic acid for Germanium pyrenaicum. Glucose was found to be the best precursor of gallic acid and phenylalanine the poorest. The results obtained were consistent with the formation of gallic acid from glucose by the shikimic acid pathway.

In contrast to the method of formation of condensed tannins, referred to previously, hydrolysable tannins (galloyl esters and gallotannins) are regarded as having their origins from esterification of gallic acid with the appropriate carbohydrate (Haslam, 1966). According to Haslam, plants differ widely in their ability to form simple or complex galloyl esters: mono-esters with D-glucose (β-D-glucogallin) and quinic acid (theogallin) occur relatively widely; but complex gallotannins with 7 or 8 galloyl groups per carbohydrate unit have a more limited distribution.

Properties of tannins

Precipitation of proteins is one of the important properties of tannins (Gayon, 1968). The active sites are the peptide bonds of the protein molecule (Haslam, 1966). In this regard, Grassmann (1937) has shown that water-soluble condensation products of urea and formaldehyde which contain peptide bonds are quantitatively precipitated by tannins. Evidence of such affinity between tannin and peptide bonds has also

been demonstrated by Gustavson (1954).

All phenols, unless sterically hindered, take part in hydrogen bonding (Thomson, 1964), and the bond formed between phenols and amides is one of the strongest types of hydrogen bonds (Flett, 1952). Phenol itself has been shown to form complexes with proteins (Gustavson, 1956a), nylon (Endres and Hörmann, 1963) and polyvinylpyrrolidone (Gustavson, 1954). In addition, many phenols are readily oxidized to highly reactive quinones (Thomson, 1964; Mason, 1955, 1959) either non-enzymatically, or by plant phenol oxidases and peroxidases. According to Thomson and Mason quinones are oxidizing agents and may oxidize essential groups of proteins. More particularly, they polymerize rapidly forming covalent bonds with protein.

The pH responses exhibited by the two types of tannins are different (Gustavson, 1956b, 1963). According to Gustavson condensed tannins are bound to other reactive groups almost independently of pH below neutral except for an indirect effect due to the swelling of collagen at pH 3 or below. The reactivity decreases rapidly above pH 8. Hydrolysable tannins, on the other hand, are bound very strongly at pH 3 to 4 but the binding decreases above pH 5.

Gustavson has shown that tannins are partially removed from all soluble and insoluble complexes with 6 to 8 M urea. Dilute alkali, or aqueous organic solvents which are capable of hydrogen bonding, such as alcohols or acetone , also break the tannin complexes (Gustavson, 1956b, 1963) but in certain cases they dissolve the substrate as well.

Tannins in nutrition

1. Effects on growth

Chang and Fuller (1964) have reported that the use of grain sorghums of high tannin content in rations for chicks resulted in growth retardation which was similar in magnitude to that caused by equivalent levels of tannic acid. These workers also noted that when choline and methionine were added to rations containing sorghum grains improved growth rates were obtained. Booth et al. (1959) have suggested that methyl donors are need for detoxification of tannic acid and its breakdown products.

In studies on the appetite depressing and growth inhibiting effects of various hydrolysable and condensed tannins, Vohra et al. (1966) found that as little as 0.5% dietary tannic acid caused a depression in the growth of chicks. Of a number of tannins studied, tannic acid proved to be the most deleterious. The growth depressing properties of tannic acid were not prevented by the addition of methionione, choline, betaine or ornithine.

2. Effects on energy utilization

In studies on the metabolizable energy value of rations containing carob meal, a rich source of tannins, (Bornstein et al., 1965; Vohra et al., 1966) found that increasing the level of carob meal in rations lowered the metabolizable energy value of the rations. When various commercially available hydrolysable and condensed tannins were used (Vohra et al., 1966), it was observed that a level of 2% tannic acid in the ration

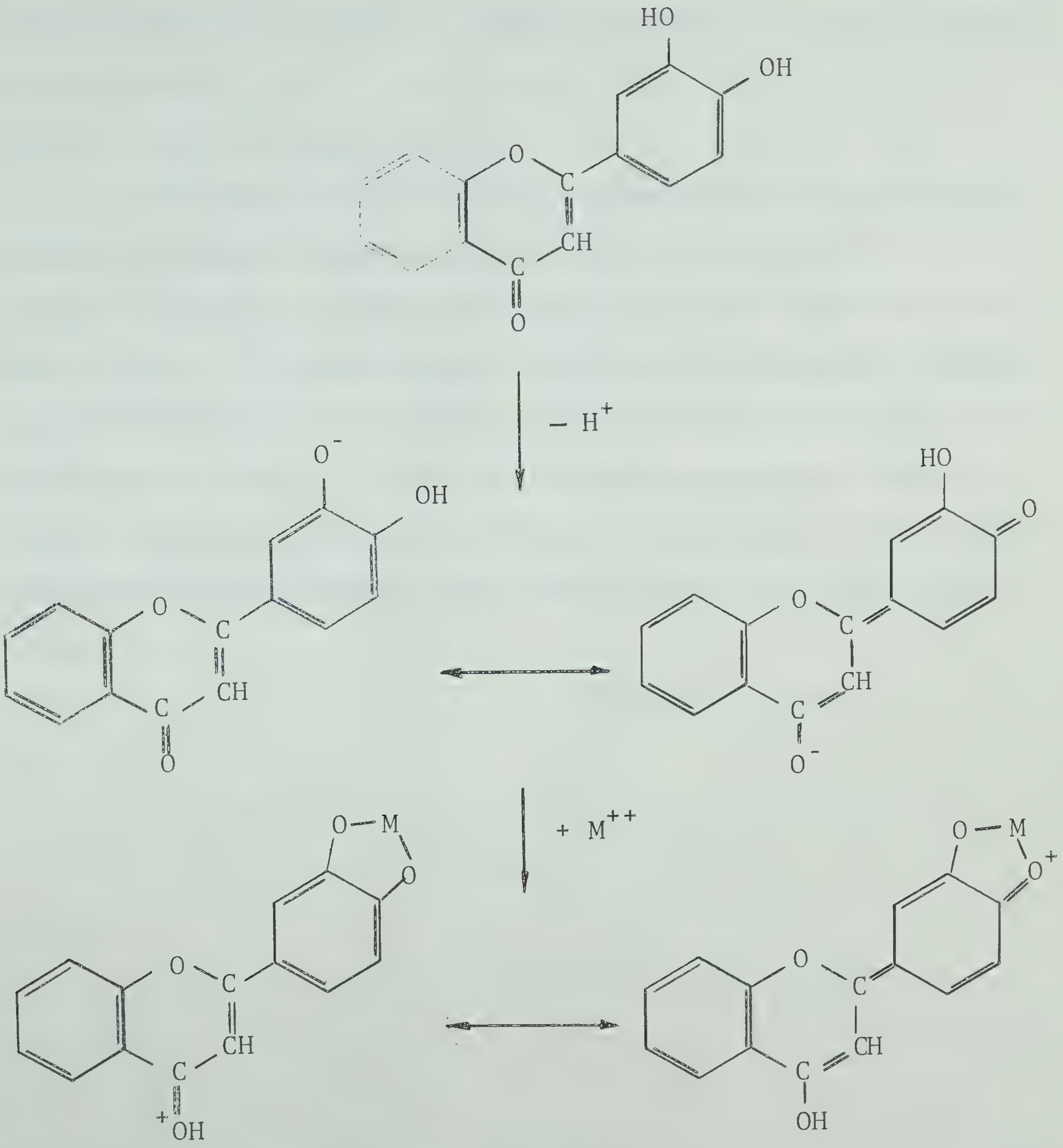
resulted in a significant decrease in the metabolizable energy content of the ration.

3. Effects on protein utilization

It has been observed that the inclusion of tannins in poultry rations results in a decrease in the utilization of protein. Chang and Fuller (1964) reported a slight depression in digestibility of protein as a result of including 1% tannin in the ration of growing chickens. They concluded that the magnitude of the growth depression, however, could not be fully accounted for on the basis of the decrease in protein digestibility noted. Bornstein et al. (1965) noted that a significant reduction in nitrogen retention occurred when rations containing high levels of carob meal, a rich source of tannins were fed. Likewise, a significant depression in protein retention by chicks as a result of feeding rations containing 2% tannic acid was reported by Vohra et al. (1966).

4. Effects on mineral utilization

The possibility of forming complexes between metals and phenolic compounds by chelation has been recognized and there is evidence that such complexes exist in nature. Jurd and Geissman (1956) observed that a great number of natural phenolic compounds contains structural groups susceptible to formation of complexes with metals. Harbone (1965) reported that phenol metal complexes participate in the coloring of plants. Jurd and Geissman (1956) suggested that formation of complexes in alkaline medium may occur as shown in Equation 4.



Equation 4. Complexes of flavan -3, 4-diol with metals

Complexes such as these could have considerable significance both with respect to the detrimental effects that they might have on the availability of minerals and to the beneficial effects that such complexes might have on reducing the

adverse effects of tannin on the utilization of other nutrients in the ration.

Tannin content of rapeseed meal

The tannin content of the prepress-solvent and solvent processed rapeseed meals were analyzed and reported to be 2.71 to 3.35% respectively with an average of 3.01% (Clandinin and Heard, 1968). It would appear therefore that rapeseed contains appreciable amounts of tannins. On the basis of the work of Vohra et al. (1966), it was concluded that rapeseed meal may contain tannins in amounts sufficient to adversely affect chick growth and metabolizable energy when the meal is fed at high levels.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Two experiments were conducted to study the effect of tannins on the nutritive value of rapeseed meal for chicks. In the first experiment tannins were extracted from rapeseed meal and the metabolizable energy content of this meal was compared with that of the unextracted rapeseed meal. The extracted tannins were added to a soybean meal containing diet and the metabolizable energy content of this diet was compared with that of the same ration without added tannins. In the second experiment, the effect of tannins in rapeseed meal on the absorbability of protein by chicks was determined by using regular and tannin-free rapeseed meals at the 30% level in rations containing soybean meal and as the sole source of supplementary protein.

1. Effect of tannins in rapeseed meal on the metabolizable energy value of rapeseed meal for chicks

Object

The purpose of this experiment was to determine whether the removal of tannins from rapeseed meal would improve its metabolizable energy value. In addition, the effect of adding the tannins removed from rapeseed meal to soybean meal on its metabolizable energy content was studied.

Experimental

One sample of rapeseed meal was used to study the effect of tannins in rapeseed meal on the metabolizable energy content of the meal for chicks. Proximate composition and tannin content of meals, determined by AOAC (1960) methods;

and oxazolidinethione and isothiocyanate content, determined by methods developed by Astwood et al. (1949) and Wetter (1955, 1957), are given in Table 1.

Tannin-free rapeseed meal, used in this study, was prepared in the following manner.

Extraction of tannins from rapeseed meal was made in a stainless steel, soxhlet-type apparatus using the procedure described by Gayon (1968). Approximately 30 litres of methanol was used as solvent for each extraction and 3-4 gms $CaCO_3$ was added to the solvent in order to decrease the acidity (Geissman, 1955). Approximately 3 kilograms of rapeseed meal were extracted for 16 hours to ensure satisfactory removal of the tannins. The extracted rapeseed meal was then oven-dried for 24 hours at 65 C, to remove methanol, and tested for tannin content. The extract was filtered immediately after extraction and concentrated, under vacuum at 30 C to about 10% of its original volume at 30 C. During concentration a sediment formed which was considered to be tannins. The sediment was separated by decanting the supernatant. The tannins remaining in solution were precipitated from the extract by adding a saturated solution of neutral lead acetate (Geissman, 1955). The precipitated tannin complexes were then separated by centrifugation and the supernatant was decanted. The precipitate was resuspended in methanol and decomposed with a stream of hydrogen sulfide. The lead was precipitated as lead sulfide, and separated by filtration. The filtrate was then concentrated under vacuum at 30 C to desired volume and

refrigerated along with the tannin sediment until they were added to the soybean meal. The supernatant from which the first lead-tannin complexes were removed was also treated with a stream of hydrogen sulfide to precipitate any lead as lead sulfide, filtered and the filtrate was added back to the extracted rapeseed meal. After reconstitution both meals were oven-dried at 65 C until methanol was completely evaporated.

Rapeseed oil was added to the extracted rapeseed meal in order to equalize the fat content of the extracted and original rapeseed meals. The extracted meal, although it contained a residue of 0.77% tannin, is referred to hereafter as tannin-free rapeseed meal.

Metabolizable energy values were determined by the method of Hill and Anderson (1958). The reference diet used for the metabolizable energy determinations is shown in Table 2.

Five rations were used in this experiment. Ration 1 was the reference ration (Table 2). Ration 2 was formulated from the reference ration by replacing 30 parts of glucose with 30 parts of rapeseed meal. Ration 3 was similar to Ration 2 except that tannin-free rapeseed meal was used in place of regular rapeseed meal. Ration 4 was made iso-nitrogenous to Ration 2 by replacing 20.72 parts of glucose with 20.72 parts of soybean meal. Ration 5 was similar to Ration 4 except that the tannins extracted from 30 parts of rapeseed meal were added to the 20.72 parts of soybean meal incorporated in this ration. All substitutions were made on

TABLE 1. - Composition of rapeseed meal and soybean meal

	Protein (Nx6.25) %	Fat %	Fibre %	Ash %	Tannin %	Isothio-cyanate mg/gm	Oxazolid-inethione mg/gm
Rapeseed meal	37.66	1.92	14.72	7.08	3.65	5.17	2.32
Soybean meal	54.50	1.28	6.46	5.96	-	-	-

Values are expressed on dry-matter basis

TABLE 2. - Composition of reference diet

Components	%
Glucose[1]	49.621
Soybean meal	35
Glycine	1
DL methionine	.5
Brewer's yeast	2.5
Dried whey	2
Mineral mix[2]	4.91
Vitamin mix[3]	.58
Soybean oil	2
Antioxidant[4]	.025
Chromic oxide mix[5]	1
Cellulose	.864
TOTAL	100

[1] Cerelose

[2] Mineral mixture supplied in milligrams per 100 grams diet: $CaHPO_4$, 2,600; $CaCO_3$, 1,300; NaCl, 600; K_2HPO_4, 220; $MgSO_4$, 115; $FeSO_4 \cdot 7H_2O$, 28; $MnSO_4 \cdot H_2O$, 33.5; $ZnCO_3$, 9.7; $CuSO_4 \cdot 5H_2O$, 0.78; KI, 0.29 and Na_2SeO_3, 0.022.

[3] Vitamin mixture supplied the following levels per 100 grams of diet: thiamine, 1.0 mg; riboflavin, 1.0 mg; calcium pantothenate, 4.0 mg; biotin, 0.04 mg; pyridoxine, 2.0 mg; niacin, 8.0 mg; folacin, 0.3 mg; menadione, 0.3 mg; vitamin B_{12}, 0.0005 mg; choline chloride, 300 mg; aureomycin, 1.0 mg; vitamin A 1,000 I.U.; vitamin D_3, 150 I.C.U.; vitamin E, 3.3 I.U.

[4] Ethoxyquin

[5] Contains: 30% chromic oxide, 70% flour

a moisture-free basis.

In the metabolizable energy determinations, male crossbred (Dominant White X White Plymouth Rock) chicks were used. They were maintained in electrically heated, thermostatically controlled battery brooders, with raised wire screen floors, in a temperature-controlled laboratory. In this experiment, male chicks were reared to one week of age on the reference diet and then alloted to groups of 10 chicks each on the basis of body weight and weight gain, equalizing both mean weight and weight distribution among the groups. Each experimental ration was fed to duplicate groups of chicks from 7 to 42 days of age. Feed and water were supplied ad libitum. The chicks were weighed at 6 weeks of age and feed consumption was recorded.

Excreta were collected at 24 hour intervals at 12, 13 and 14 days of age and again at 40, 41 and 42 days of age. The three-day collections from each group were pooled and kept at -25 C until analyzed. Chromic oxide was incorporated in all diets at a level of approximately 0.3% as an index substance in order to eliminate the need for quantitative measurements of feed intake. The methods of processing excreta and of conducting chemical analyses for moisture, nitrogen, combustible energy and chromic oxide and of computing metabolizable energy from these data have been described previously (Hill and Anderson, 1958; Hill et al., 1960). Glucose, the reference material used in the diets for chicks, was assumed to have a metabolizable energy value of 3.64 kcal per gram (Anderson

et al., 1958).

At six weeks of age, 4 chicks from each group were killed, and their thyroid glands were removed and weighed. Thyroid-to-body-weight ratios were calculated for each chick.

Results and Discussion

The results, summarized in Table 3, show the metabolizable energy values of rapeseed meal and soybean meals for chicks determined during the 2nd week and 6th week of life after the chicks had been fed the test diets for either 5 or 33 days.

Analysis of variance (Steel and Torrie, 1960) showed that there was a highly significant ($P < 0.01$) difference in the energy content of the protein supplements, however, energy values were not affected by the length of time that chicks were fed the test rations.

Removal of the tannins from rapeseed meal significantly increased ($P < 0.01$) its metabolizable energy content at both the first and second collection. The metabolizable energy of the rapeseed meal increased from 1,171 to 1,844 kcal per kg when the metabolizable energy was determined during the 2nd week and from 1,253 to 1,766 when the metabolizable energy was determined during the 6th week of age. In the case of the soybean meal, the addition of tannins significantly lowered ($P < 0.01$) the metabolizable energy content of the meal from 2,749 to 2,536 kcal per kg at the first collection but gave a non-significant reduction, ($P < 0.05$) from 2,759 to 2,613 kcal per kg at the second collection.

TABLE 3. - Effect of tannins on the metabolizable energy value of rapeseed meal and soybean meal for chicks

Treatments	Interval on treatments, days	
	7 to 14[1]	7 to 42[1]
	kcal/kg	kcal/kg
Rapeseed meal	1,171	1,253
Rapeseed meal - tannins	1,844	1,766
Soybean meal	2,749	2,759
Soybean meal + tannins	2,536	2,613

[1] Collections for metabolizable energy determinations were made on the last 3 days of each experimental period.

[2] Values are expressed on a dry-matter basis and represent the average of duplicate groups.

The increased metabolizable energy content of the tannin-free rapeseed meal was probably a result of better nutrient utilization perhaps because of enchanced enzyme activity. Numerous studies have shown that tannins affect enzyme activity in plants and animals. Hulme and Jones (1962) found that tannins were responsible for the low activity of apple mitochondrial enzymes. Enzyme activity increased after the addition of polyvinylpyrrolidone which is known to bind vegetable tannins. Blumenberg and Kessler (1964) reported the inhibition of lactate dehydrogenase, glutamate-oxalate transaminase and alkaline and acid phosphatases by tannin solutions. Goldstein and Swain (1965) who made an extensive study of the inhibition of β-glucosidase by tannic acid and condensed tannin fractions from bark of wattles, noted that the inhibition was non-competitive. Tagari et al. (1965) observed an inhibition of cellulytic and proteolytic activity in an artificial rumen by extracts from carobs. Other authors, Loomis and Battaile (1964) Sanderson (1965), observed that the removal of polyphenols from enzyme preparations of plant origin greatly enchanced the enzyme activity. Tamir and Alumot (1969) determined the degree of inhibition of trypsin, α-amylase and lipase by condensed carob tannins and compared with that caused by a glucoside of m-digallic acid.

Depression of the metabolizable energy content of soybean meal by added tannins was significant in one instance but the extent of reduction of the metabolizable energy value

was not of the magnitute expected on the basis of the improvement noted in rapeseed meal from which the tannins were removed. This may have been due to alteration in the composition of tannins during the extraction procedure. Evidence for such alterations has been reported by Haslam (1966).

Body weight, thyroid-to-body-weight ratios and calories consumed per gram gain to six weeks of age are presented in Table 4. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the growth data showed that the chicks fed the rapeseed meal-containing rations were significantly lower ($P < 0.01$) in body weight at 42 days of age than those fed the soybean meal-containing rations. In this connection, however, it should be pointed out that the rapeseed meal and soybean meal-containing diets were not isocaloric. Hence a similar growth response was not anticipated. While slight differences in thyroid-to-body-weight rations were noted these were not significant ($P < 0.05$).

Caloric efficiencies, calculated in order to determine whether energy utilization was affected by treatment, revealed on analysis, no significant differences between treatments ($P < 0.05$).

Summary

A study was made on the effect of tannins in rapeseed meal on the metabolizable energy of the meal. Results showed that removing the tannins from rapeseed meal increased the metabolizable energy value or rapeseed meal for chicks from 1,171 to 1,844 kcal per kg and from 1,253 to 1,766 kcal per

TABLE 4. - Growth, thyroid-to-body-weight ratios and caloric efficiency of chicks fed experimental diets

	Body wt 6 wk, g	Mg thyroid per 100 g body wt	Calories consumed per g gain		
Interval on treatments, days	7 to 42	7 to 42	7 to 14	7 to 42	14 to 42
Reference	985	6.95	4.48	6.30	6.58
Rapeseed meal	901	10.98	4.46	6.23	6.60
Rapeseed meal - tannins	967	8.73	4.36	6.15	6.52
Soybean meal	990	8.95	3.91	6.23	6.65
Soybean meal + tannins	982	9.54	3.95	6.32	6.64

All values averages of duplicate groups.

kg when the determinations were done at 2 week and 6 weeks of age respectively.

Addition of tannins extracted from rapeseed meal to soybean meal resulted in a reduction in its metabolizable energy value.

The presence or absence of tannins had no significant effect on average body weight or caloric efficiencies of chicks fed rations containing either rapeseed meal or soybean meal.

2. Effect of tannins in rapeseed meal on the absorbability of protein in rapeseed meal by chicks

Object

The results of the previous experiment indicated that the extraction of the tannins from rapeseed meal increased its metabolizable energy content. On the assumption that the improvement in metabolizable energy value was the result of better nutrient utilization, studies were conducted to determine whether or not improved absorbability of nitrogen as a result of removing tannins from rapeseed meal was a contributing factor to the improvement in its metabolizable energy value.

Experimental

In this experiment the absorption of nitrogen by chicks, from rations containing rapeseed meal or tannin-free rapeseed meal as the sole source of protein or as a replacement for part of the glucose in the reference diet, was studied using the nitrogen remaining in the terminal fifth

segment of the small intestine as the measure of unabsorbed nitrogen as described by Lodhi et al. (1970b).

The composition of the rations used in this study is shown in Table 5.

Rations 1, 2 and 3 were the same as three of the rations used in the first experiment. In rations 4 and 5 rapeseed meal and tannin-free rapeseed meal served as the sole source of dietary protein. Rations 4 and 5 were formulated to contain 14.1 kcal of metabolizable energy per gram protein in diets. Chromic oxide was incorporated in all rations at a level of approximately 0.8% as an index substance.

In this study crossbred (Dominant White X White Plymouth Rock) chicks of mixed sexes were fed to one week of age on Ration 1. At the end of the pre-experimental period, five male and five female chicks were allotted the ten comparable groups according to the method described by McKittrick (1947). During the ensuing three weeks the ten groups were continued on Ration 1. At the end of this time duplicate groups were placed on each of the five rations shown in Table 5. Management of the chicks was similar to that described in the first experiment.

In order that metabolizable energy values could be determined, excreta were collected from each group of chicks on the 40th, 41st and 42nd days. The methods for collecting, holding and processing excreta and for conducting chemical analyses for moisture, nitrogen, combustible energy and chromic oxide were similar to those referred to in the first

TABLE 5. - Composition of experimental rations

Components	Ration no.				
	1	2	3	4	5
	%	%	%	%	%
Glucose	49.621	19.621	19.621	17.506	25.01
Soybean meal	35	35	35	-	-
Glycine	1	1	1	1	1
DL methionine	0.5	0.5	0.5	0.5	0.5
Rapeseed meal	-	30	-	56.615	-
Rapeseed meal, tannin-free	-	-	30	-	55.565
Brewer's yeast	2.5	2.5	2.5	-	-
Dried whey	2	2	2	-	-
Mineral mix[2]	4.91	4.91	4.91	4.91	4.91
Vitamin mix[3]	0.58	0.58	0.58	0.58	0.58
Soybean oil	2	2	2	17.864	11.41
Antioxidant[4]	0.025	0.025	0.025	0.025	0.025
Chromic oxide mix[5]	1	1	1	1	1
Cellulose	.864	.864	.864	-	-
TOTALS	100	100	100	100	100

[1] Cerelose

[2] Mineral mixture supplied in milligrams per 100 grams diet: $CaHPO_4$, 2,600; $CaCO_3$, 1,300; NaCl, 600; K_2HPO_4, 220; $MgSO_4$, 115; $FeSO_4 \cdot 7H_2O$, 28; $MnSO_4 \cdot H_2O$, 33.5; $ZnCO_3$, 9.7 $CuSO_4 \cdot 5H_2O$, 0.78; KI, 0.29 and Na_2SeO_3, 0.022.

[3] Vitamin mixture supplied the following levels per 100 grams of diet: thiamine, 1.0 mg; riboflavin, 1.0 mg; calcium pantothenate, 4.0 mg; biotin, 0.04 mg; pyridoxine, 2.0 mg; niacin, 8.0 mg; folacin, 0.3 mg; menadione, 0.3 mg; vitamin B_{12}, 0.0005 mg; choline chloride, 300 mg; aureomycin , 1.0 mg; vitamin A 1,000 I.U.; vitamin D_3, 150 I.C.U.; vitamin E, 3.3 I.U.

[4] Ethoxyquin

[5] Contains: 80% chromic oxide, 20% flour

experiment. The metabolizable energy values for rapeseed meal and tannin-free rapeseed meal were calculated from the determined metabolizable energy values of the respective rations by subtracting the energy contributed by the glucose and soybean oil included in the rations. In these calculations glucose and soybean oil were assigned metabolizable energy values of 3.64 (Anderson et al., 1958) and 9.21 (Renner and Elcombe, 1964) kcal per gram respectively.

At six weeks of age, the chicks were killed with chloroform and their digestive tracts were removed immediately. The small intestine, from the proximal end of the duodenum to the junction of the caeca and colon, was divided into five segments. The contents of the terminal fifth segment of the small intestine were removed using distilled water and gentle pressure. This was done as soon as possible after killing to avoid, in so far as possible, shedding of epithelial tissue into the gut. The intestinal contents thus obtained were freeze-dried and analyzed for chromium and nitrogen using the methods described by Hill and Anderson (1958). The absorbability of nitrogen was determined by comparing the ratio of nitrogen to chromic oxide in the feed to the ratio of nitrogen to chromic oxide in the content of the terminal fifth of the small intestine.

Results and Discussion

Summarized in Table 6 are data showing the apparent absorbability of nitrogen in rapeseed meal and soybean meal for 6 week old chickens. When soybean meal and rapeseed meal served as the sole source of protein in the ration the

TABLE 6. - Absorbability of nitrogen

Ration no.	Treatment	Apparent nitrogen absorption
		%
1	Soybean meal, sole source of protein	85.2
2	Rapeseed meal 30%	62.6
3	Rapeseed meal, tannin-free, 30%	64.2
4	Rapeseed meal, sole source of protein	79.7
5	Rapeseed meal, tannin-free, sole source of protein	80.4

TABLE 7. - Metabolizable energy values

Ration no.	Treatment	Metabolizable energy, 6 wk, kcal per kg
1	Soybean meal, sole source of protein	2,753
2	Rapeseed meal 30%	1,237
3	Rapeseed meal, tannin-free, 30%	1,795
4	Rapeseed meal, sole source of protein	1,761
5	Rapeseed meal, tannin-free, sole source of protein	1,803

absorbabilities of nitrogen were 85.2 and 79.7%, respectively. Analysis of variance (Steel and Torrie, 1960) showed that the apparent absorbability of nitrogen in rapeseed meal was significantly lower than in soybean meal ($P < 0.01$). These findings are in agreement with those of Lodhi et al. (1970b).

The data also show that the absorbability of the nitrogen in rapeseed meal was lower when it served as a partial source of protein than when it provided all of the protein in the ration, with values of 62.6 and 79.7%, respectively. The results agree closely with the findings of Lodhi et al. (1970b).

Apparent nitrogen absorption did not appear to be increased by the extraction of tannins from rapeseed meal. There was no significant difference in absorbability of nitrogen from tannin-containing and tannin-free rations regardless of whether rapeseed meal provided a part or all of the protein in the ration.

Metabolizable energy values of rapeseed meal and tannin-free rapeseed meal for 6 week old chicks are presented in Table 7.

The metabolizable energy content of soybean meal was significantly higher ($P < 0.01$) than either regular or tannin-free rapeseed meal. The data show that the metabolizable energy content of rapeseed meal was significantly lower ($P < 0.01$) when meal was substituted, weight for weight, for glucose in a ration containing soybean meal rather than when it served as the sole source of nitrogen. These results agree with the finding of Lodhi et al. (1969, 1970a).

TABLE 8. - Effect of rapeseed meal and soybean meal as protein sources on body weight and caloric efficiency

Ration no	Treatment	Body wt 6 wk	Calories consumed per g gain, 28-42 days
1	Soybean meal, sole source of protein	928	7.72
2	Rapeseed meal 30%	860	7.76
3	Rapeseed meal, tannin-free, 30%	871	8.00
4	Rapeseed meal, sole source of protein	882	8.31
5	Rapeseed meal, tannin-free sole source of protein	886	7.71

Removal of tannins from the rapeseed meal significantly ($P < 0.01$) improved the metabolizable energy value of the rapeseed meal when it served as a partial source of protein in the ration (1,795 vs 1,237 kcal per kg) but did not appear to affect its metabolizable energy value when it served as the sole source of protein in the ration (1,803 vs 1,761 kcal per kg). Explanation of this result must await further studies.

Analysis of variance of the growth data (Table 8) showed no significant differences ($P < 0.05$) between the treatments receiving regular or tannin-free rapeseed meal. Calories consumed per gram of gain were significantly higher ($P < 0.05$) in the group fed regular rapeseed meal as the sole source of nitrogen than in the groups fed soybean meal or tannin-free rapeseed meal but efficiency of energy utilization was not affected when regular and tannin-free rapeseed meals were used in combination with soybean meal.

Summary

A study was conducted on the effect of tannins on nitrogen absorption and the metabolizable energy value of rapeseed meal.

Nitrogen absorption from rapeseed meal and tannin-free rapeseed meal were 62.6% and 64.2% respectively when fed as part of the nitrogen source and 79.7% and 80.4% respectively when fed as sole source of nitrogen in the ration. Hence the presence of tannins in rapeseed meal apparently had no appreciable effect on nitrogen absorption.

The removal of tannins from rapeseed meal resulted in

a marked increase in metabolizable energy content when rapeseed meal served as a partial source of protein in the ration but had no effect on metabolizable energy when it provided all of the protein in the ration.

GENERAL DISCUSSION

The results of the foregoing appear to indicate that the presence of tannins in rapeseed meal affects the metabolizable energy content of the meal. When tannins were removed from the meal by extraction with methanol, the metabolizable energy content of the meal was increased in comparison with the unextracted meal. The fact that the addition of extracted tannins to soybean meal resulted in a decrease in the energy content of the meal seems to support the view that tannins are, in part, responsible for the low metabolizable energy content of rapeseed meal.

Since metabolizable energy of a feed is derived from available carbohydrate, protein and fat, it would seem logical that factors causing a depression in metabolizable energy might act by reducing the utilization of one or all of these nutrients. The results of the study on the effect of tannins of the absorbability of the nitrogen in rapeseed meal, however, indicated that the presence or absence of tannins had no effect on the absorbability of nitrogen. Furthermore, this finding that the metabolizable energy content of rapeseed meal was unaffected by the removal of tannins when it served as the sole source of protein casts some doubt on the conclusion that the presence of tannins in rapeseed meal reduces its metabolizable energy value.

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